Interactions of Phaeocystis sp. with organic compounds and the microbial foodweb

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Chapter 6

Phaeocystis colony mucus components and the importance of calcium ions for colony stability

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PHAEOCYSTIS COLONY MUCUS COMPONENTS AND THE IMPORTANCE OF CALCIUM IONS FOR COLONY STABILITY

ABSTRACT: *The composition and properties of Phaeocystis colony mucus are still largely obscure. In this study some components of the mucus were identified with the use of a specific staining technique and the role of Ca^{2+} and other cations as binding agent was investigated. Also, the effect of Ca^{2+} concentration on colony formation in batch cultures was studied. Colonies of Phaeocystis sp. were stained with alcian blue at two different pH values. This revealed that the colony mucus contained both carboxylated and sulfated polymers. Incubation of colonies in medium lacking one or more cations showed that calcium and magnesium ions were essential for the gelling of colony mucus, while potassium ions had no influence. The percentage colony cells formed by Phaeocystis in batch cultures was reduced in medium with calcium concentrations below 2.5 mmol l^{-1}. No colonies were formed in medium with calcium concentrations below 1.5 mmol l^{-1}. Growth rate was not dependent on calcium concentration. It is suggested that under natural conditions Phaeocystis colony firmness and morphology might depend on the composition of mucus polymers.*

Introduction. In the marine environment mucus production is known in bacteria (Decho 1990), macro-algae (Boney 1981) and several groups of micro-algae like diatoms (Decho 1990), green algae (Crayton 1982) and Prymnesiophyceae (Painter 1983). The composition of mucus produced by these groups is often rather complex, consisting of heteropolymeric chains containing a wide variety of simple sugars, aminosugars, uronic acids, sulfated- or phosphated sugars, amino acids, etcetera. The gelling capacity of mucus depends on the binding of negatively charged groups in the molecule (mostly carboxyl-groups) with cations (mostly Ca^{2+}). In this way ionic bridges are formed between polymer strands. The number of ionic bridges formed in a polymer depends on the amount of anionic groups and the steric arrangement of these groups in the molecule (Kohn et al. 1968). Colony forming algae like Volvocaceae produce mucus with high amounts of carboxyl- and sulfate-groups (Crayton 1982).

The alga Phaeocystis sp. is an important component of the phytoplankton of several marine ecosystems such as the North Sea and the Arctic and Antarctic oceans (Barnard et al. 1984, Palmisano et al. 1986, Cadée & Hegeman 1986, Lancelot et al. 1987, Gibson et al. 1990, Wassmann et al. 1990). Phaeocystis forms colonies consisting of mucus in which cells are randomly distributed. The colonies are spherical or elongated and reach sizes of up to 5 mm in diameter containing over 10,000 cells (Rouseau et al. 1990). During bloom situations in the North Sea, when Phaeocystis cell number often exceeds 50 10^6 l^{-1} (Cadée

& Hegeman 1986), colony mucus may represent 50% or more of total phytoplankton biomass (Rouseau et al. 1990). The formation of foam layers on beaches following Phaeocystis blooms (Bätje & Michaelis 1986) suggests that the mucus is not easily decomposed either (photo)chemically or by bacteria. The composition of Phaeocystis mucus is as yet largely unknown. The research that has been done yielded contradictory results. Guillard & Hellebust (1971) found that the mucus of two Phaeocystis strains consisted of oligo- and polysaccharides of heterogenous composition. Painter (1983) gave some preliminary results of the analysis of an impure bloom of Phaeocystis, showing that the mucus might be a very complex, soluble proteoglycan. Lancelot et al. (1991) mentioned that the mucus is a polysaccharide, mainly composed of glucose units. Knowledge of mucus composition and properties is essential for understanding the role of Phaeocystis in the ecosystem, since it may help to explain the processes of Phaeocystis colony formation and -growth, as well as diffusion inside colonies, aggregation and sedimentation of colonies and the flux of mucus carbon to the microbial foodweb. This study presents a further step in elucidating the composition and properties of the colonial mucus of Phaeocystis.

Materials and methods. All experiments were performed with axenic Phaeocystis sp. (strain K) isolated from the Dutch Wadden Sea. This strain formed globosa-type colonies (Jahnke 1989). Phaeocystis was grown in 1 l. serum bottles incubated on a rolling device at 10 °C and a light intensity of ±40 µE m^{-2} s^{-1} in a 14:10 L:D cycle. The artificial seawater medium described by Veldhuis & Admiraal (1987) was used for culturing and for all experiments. The medium had nutrient concentrations of 4 µmol l^{-1} PO_4^{3-} and 70 µmol l^{-1} NO_3^-, while the Ca^{2+} concentration was 3.6 mmol l^{-1}. Under these conditions Phaeocystis predominantly formed large colonies, suitable for the experiments described below. The presence of carboxylated and sulfated polymers in mucus of living Phaeocystis colonies was investigated with the use of alcian blue, a cationic copper phtalocyanine dye, which is specific for these poly-anionic groups (Scott et al. 1964, Ramus 1977). Differentiation between carboxylated and sulfated polysaccharides was possible since the dye binds to both types at pH 2.5, and to sulfated polymers only at pH 0.5 (Crayton 1982). Alcian blue does not bind to phosphate groups at these pH values (Scott et al. 1964). The staining method used by Crayton (1982) was adapted for use in seawater medium. To obtain the different pH values in the reaction mixture 0.1 % (w/v) alcian blue was made in either 0.5 N HAc (for pH 2.5) or in 0.5 N HCl (for pH 0.5). The alcian blue stocks were prepared in a solution containing the major salts of the sea water medium used for culturing Phaeocystis in order to prevent osmotic shock of the cells during treatment. For staining of colonies the alcian blue stocks were mixed 5 parts to 2 with

concentrated colony suspensions in glass tubes. The mixtures were kept at room temperature at least 15 minutes. Thereafter they were diluted tenfold with seawater in order to make the colonies visible. Colonies were picked out with pipettes, transferred to microscope chambers and observed with an inverted microscope. Control experiments showed that alcian blue did not bind to living single cells of Phaeocystis, indicating that staining was specific for mucus. Pieces of agar gel (a polymer containing both carboxylated and sulfated groups) that were stained using the same method as for colonies showed a strong blue colour at both pH values, indicating that the seawater medium did not affect the staining reaction. Also, Crayton (1982) found that addition to the reaction mixture of up to 0.5 M NaCl did not affect binding of alcian blue to polymers at both pH values.

Since cations were expected to have an important function in the gelling of colony mucus, the effect of omission of one or more cations from the medium on colony cohesion was examined. For this purpose seawater medium was prepared in which K^+, Ca^{2+}, Mg^{2+}, a combination of two of those, or all three were lacking. The omitted ion was replaced by Na^+ in order to maintain approximately the same salinity in the medium. Triple 5 ml portions of these media and of complete medium (control) were distributed over sterile, translucent culturing plates, containing 25 chambers each. Healthy colonies were concentrated from a Phaeocystis culture growing in standard medium and small aliquots (0.1 to 0.25 ml) of this concentrate were transferred to the different media in the chambers. The plates were incubated for one week at 10 °C and low light intensity and colonies were checked regularly using inverted microscopy.

The effect of different calcium concentrations on colony formation during growth was studied in batch culture experiments. For these experiments calcium-free medium was inoculated with Phaeocystis and distributed in 200 ml portions over 250 ml serum bottles. To each bottle a different amount of a $CaCl_2$ stock solution was added, to final calcium concentrations ranging between 0.175 and 7.5 mmol Ca l^{-1}. The bottles were incubated under standard conditions as described above. Growth and colony formation in each culture was followed in time. Cell counts were performed according to van Boekel & Veldhuis (1990). In all experiments cultures were examined regularly for bacterial contamination with fluorescence microscopy after staining of samples with Hoechst dye no. 33258 (Paul, 1982). No bacterial contamination was detected during the experiments.

Results and discussion. Treatment of Phaeocystis sp. colonies with alcian blue at pH 2.5 resulted in a strong blue staining of the colony mucus (visible as the grey tint of the colony mucus in Fig. 1b compared with the translucent appearance of the mucus in the control colony in Fig. 1a). At pH 0.5 staining of the mucus was less but still evident (in

Fig. 1c mainly visible as the darker colony membrane compared with the control). These results indicate that Phaeocystis colony mucus contains both carboxylated and sulfated polymers. Preliminary results of NMR analysis of purified mucus from Phaeocystis cultures and field samples indicate that the mucus is of complex composition. The NMR spectra of Phaeocystis mucus show no resemblance with spectra of known algal or other polymers (H. Huizing, personal communication). This is in accordance with the results of Painter (1983) who found hemiester sulfates and residues of galacturonic acid, together with glucosamine and a number of saccharides, in extracts of a natural Phaeocystis bloom. Polymer production also occurs in other members of the class Prymnesiophyceae to which Phaeocystis belongs. Emiliania huxleyi and Hymenomonas carterae both produce a complex glycan containing uronic acids and various saccharides (de Jong 1975, Painter 1983). These polymers have a strong calcium binding capacity and are involved in the coccolith formation.

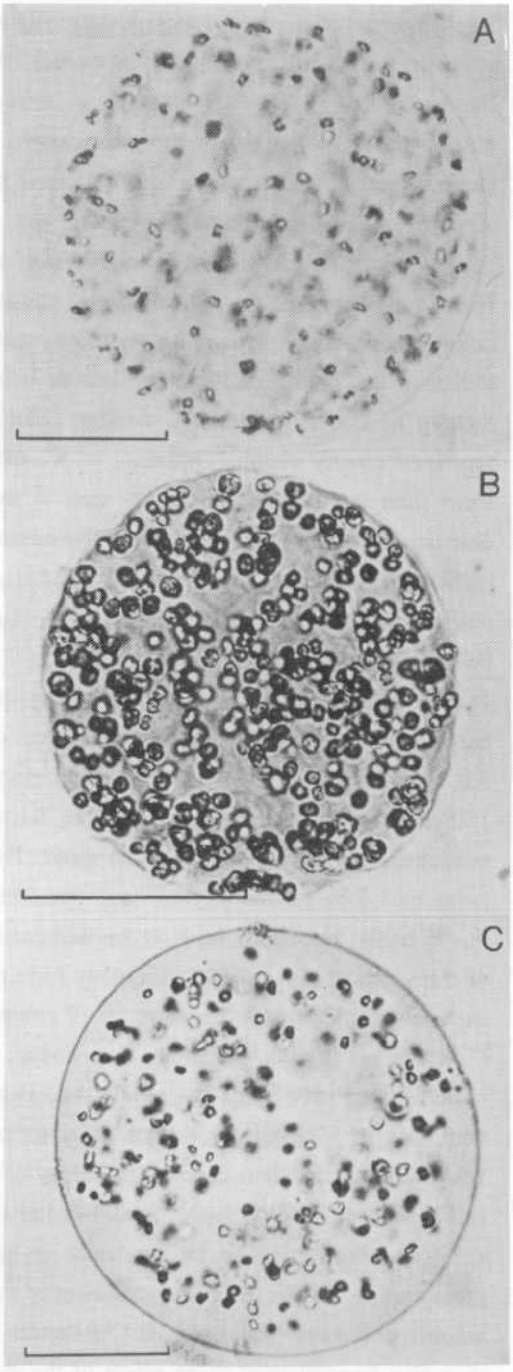

Figure 1.
Alcian blue staining of colonies of Phaeocystis sp.: unstained colony (a), colony stained at pH 2.5 (b), colony stained at pH 0.5 (c)
Scale = 50 µm

The polymer produced by Phaeocystis might very well resemble these polymers both in structure and in calcium-binding properties (see below).

Incubation of Phaeocystis colonies in media lacking one or more cations showed that mucus gelling depended on bivalent cations (Ca^{2+}, Mg^{2+}). Colonies incubated in medium lacking one or both of these ions lost their shape and fell apart within one day. The first sign of this disintegration process was the disappearance of what is called the colonymembrane after about 4 hours. This 'membrane' (encircling the colony as visible in Fig. 1) probably is an optical effect, caused by the difference in optical properties of colony mucus and surrounding seawater medium. The disappearance of the membrane thus indicated the fading of the sharp border between colony mucus and medium. In medium lacking K^+ and in the control medium colonies remained intact and the colony membrane remained clearly visible. Omission of K^+ and Mg^{2+} caused cell death in the colonies after short time but colony cells grew well in medium lacking Ca^{2+}. Prolonged incubation of colonies in calcium-free medium in the culture plates resulted in the formation of lumps of cells loosely packed in mucus. These lumps could easily be dispersed by moving the culture plate. Apparently the cells still produced mucus, but the absence of calcium made colony formation impossible.

In another experiment Phaeocystis colony formation during growth was further tested in batch cultures with calcium concentrations of 0.175, 0.35, 1.25, 1.5, 1.75, 2.0, 2.5, 3.75, 5.0, and 7.5 mmol Ca l^{-1} (Table 1). In cultures with calcium concentrations of 0.35 and 1.25 mmol l^{-1} only single cells were found. Colonies were formed when the calcium concentration was 1.5 mmol l^{-1} or more. The percentage of colony cells increased in the range of 1.5 to 2.5 mmol Ca l^{-1} and remained approximately the same above 2.5 mmol Ca l^{-1}. Probably, the upper limit to the percentage of colony cells was determined by the rate of transition of single cell into colony-forming cell in the cultures. Growth rates during the exponential phase were the same in all cultures except in the culture with 0.175 mmol Ca l^{-1} where no growth occurred. It is known that gel firmness depends on the number of ionic bridges serving as cross-links between polymeric chains (Decho 1990). It is shown here that in Phaeocystis calcium and magnesium serve as intermediates in these ionic bridges. Other bivalent cations may also be involved. Davidson & Marchant (1987) and Lubbers et al. (1990) found accumulation of Mn in Phaeocystis colonies. Lubbers et al. (1990) explained this to be the result of high pH values inside the colony which led to precipitation of Mn. It is known however that heavy-metal ions bind very well with poly-anionic polymers (Smidsrød & Christensen 1991). The observed Mn accumulation might thus also be explained as an effect of the mucus composition of Phaeocystis colonies. The results of the batch cultures experiment suggest that at calcium concentrations below

Table 1. Percentage colony cells and growth rate of Phaeocystis sp. in batch cultures with different calcium concentrations in the medium.

Calcium concentration (mmol l^{-1})	% colony cells in culture	growth rate (day^{-1})
0.175	--	--
0.35	0	0.54
1.25	0	0.57
1.5	6	0.55
1.75	17	0.48
2.0	45	0.56
2.5	71	0.51
3.75	87	0.51
5.0	86	0.56
7.5	73	0.61

2.5 mmol l^{-1} formation and growth of colonies of Phaeocystis is hampered due to low gel firmness caused by calcium undersaturation. In cultures with 2.5 mmol Ca l^{-1} or more gel firmness does not determine colony stability. In natural seawater the calcium concentration is approximately 10.0 mmol l^{-1} (Kennish 1989). Evidently, in natural Phaeocystis colonies undersaturation of the calcium binding sites in the mucus will not occur easily.

Gel firmness of the mucus will also depend on its monomer composition. The ability to change mucus composition might be valuable to Phaeocystis. An increase of the amount of carboxylated and/or sulfated polymers in the mucus might yield a stronger gel that is more resistant to turbulence or penetration by predators. A weaker gel might be advantageous in situations where fast diffusion of nutrients inside the colony is preferable. Also, differences in Phaeocystis colony morphology, as found in the North Sea and Arctic waters (Baumann & Jahnke 1986, Rick & Aletsee 1989) and in Antarctic waters (A. Buma personal communication), may be the result of differences in colony mucus composition.

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